

We claim:

1. A method for producing a cytokine in a plant host system wherein said plant host system has been transformed with a chimeric nucleic acid sequence encoding said cytokine, comprising the step of:

cultivating said transformed plant host system under the appropriate conditions to result in the expression of said cytokine in said plant host system

wherein said cytokine accumulates to a level greater than 1% of the total soluble protein in a sample of said plant host system.

2. The method of claim 1, further comprising the step of purifying said expressed cytokine from said plant host system.

3. The method of claim 1, wherein said expressed cytokine is free from amino acid modifications.

4. The method of claim 3, wherein said amino acid modification comprises the addition of hydroxyproline to said cytokine.

5. The method of claim 1, wherein said cytokine is free of novel glycosylation.

6. The method of claim 1, wherein said chimeric nucleic acid sequence comprising:
a first nucleic acid sequence capable of regulating the transcription in said plant host system of a second nucleic acid sequence wherein said second nucleic acid sequence encodes a signal sequence is linked in reading frame to a third nucleic acid sequence encoding a cytokine.

7. The method of claim 6, wherein said nucleic acid sequence further comprises a fourth nucleic acid sequence linked in reading frame to the 3' end of said third nucleic acid sequence.

8. The method of claim 7, wherein said fourth nucleic acid sequence encodes a "KDEL" amino acid sequence.

9. The method of claim 6, wherein said nucleic acid sequence capable of regulating transcription comprises a plant active promoter.

10. The method of claim 6, wherein said second nucleic acid sequence is capable of targeting said cytokine to a sub-cellular location within a plant host system.

11. The method of claim 10, wherein said sub-cellular location comprises the cytosol.

12. The method of claim 10, wherein said sub-cellular location comprises a plastid.

13. The method of claim 10, wherein said sub-cellular location comprises the endoplasmic reticulum.

14. The method of claim 6, wherein said second nucleic acid sequence comprises a sufficient portion of ubiquitin.

15. The method of claim 14, wherein said ubiquitin comprises an ubiquitin monomer derived from yeast.

16. The method of claim 15, wherein said ubiquitin comprises an ubiquitin monomer of potato ubiquitin gene 3.

17. The method of claim 6, wherein said second nucleic acid sequence comprises a sufficient portion of an oleosin protein to provide targeting within said plant host system.

18. The method of claim 17, wherein a nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by enzymatic or chemical means is included between said second nucleic acid sequence encoding said oleosin protein and the third nucleic acid sequence encoding a cytokine.

19. The method of claim 18, wherein a nucleic acid encoding said oleosin protein is derived from soy.

20. The method of claim 1, wherein said cytokine is a member of the cytokine superfamily selected from the group consisting of TGF-beta, PDGF, EGF, VEGF; chemokines; and FGFs.

21. The method of claim 20, wherein said cytokine comprises hGH.

22. The method of claim 20, wherein said cytokine comprises G-CSF.

23. A plant host system that has been transformed with a chimeric nucleic acid sequence wherein said chimeric nucleic acid sequence comprises:

a first nucleic acid sequence capable of regulating the transcription in said plant host system of a second nucleic acid sequence wherein said second nucleic acid sequence encodes a signal sequence that is linked in reading frame to a third nucleic acid sequence encoding a cytokine.

24. The method of claim 23, wherein said nucleic acid sequence further comprises a fourth nucleic acid sequence linked in reading frame to the 3' end of said third nucleic acid sequence.

25. The method of claim 24, wherein said fourth nucleic acid sequence encodes a "KDEL" amino acid sequence.

26. The plant host system of claim 23, wherein said first nucleic acid sequence comprises a plant active promoter.

27. The plant host system of claim 23, wherein said signal sequence capable of targeting said cytokine to a sub-cellular location within said plant host system.

28. The plant host system of claim 23, wherein said signal sequence is capable of targeting said cytokine to the cytosol of said plant host system.

29. The plant host system of claim 23, wherein signal sequence is capable of targeting said cytokine to a plastid within said plant host system.

30. The plant host system of claim 23, wherein said signal is capable of targeting said cytokine to the endoplasmic reticulum located within said plant host system.

31. The plant host system of claim 23, wherein said signal sequence comprises ubiquitin.

32. The method of claim 31, wherein said ubiquitin comprises an ubiquitin monomer derived from yeast.

33. The method of claim 31, wherein said ubiquitin comprises an ubiquitin monomer of potato ubiquitin gene 3.

34. The plant host system of claim 23, wherein said signal sequence comprises a sufficient portion of oleosin to target said cytokine within said plant host system.

35. The plant host system of claim 34, wherein a nucleic acid encoding said oleosin is derived from soy.

36. The plant host system of claim 23, wherein a nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by enzymatic or chemical means is included between said signal sequence and said third nucleic acid sequence encoding a cytokine.

37. The plant host system of claim 36, wherein said cleavable amino acid sequence comprises enterokinase.

38. The plant host system of claim 36, wherein said signal sequence comprises a sufficient portion of oleosin protein to target said cytokine within said plant host system.

39. The plant host system of claim 38, wherein a nucleic acid sequence encoding said oleosin protein is derived from soy.

40. The plant host system of claim 23, wherein cultivating said plant host system under the appropriate conditions results in the expression of said cytokine.

41. The plant host system of claim 40, wherein said expressed cytokine is purified from said plant host system.

42. The plant host system of claim 40, wherein said expressed cytokine is free from amino acid modifications.

43. The plant host system of claim 42, wherein said amino acid modification comprises the addition of hydroxyproline to said cytokine.

44. The plant host system of claim 40, wherein said expressed cytokine is free from novel glycosylation.

45. The plant host system of claim 23, wherein said expressed cytokine is a member of the cytokine superfamily selected from the group consisting of TGF-beta, PDGF, EGF, VEGF; chemokines; and FGFs.

46. The plant host system of claim 45, wherein said expressed cytokine comprises hGH.

47. The plant host system of claim 46, wherein the N-terminus of said expressed hGH is identical to authentic N-terminus of hGH.

48. The plant host system of claim 45, wherein said expressed cytokine comprises G-CSF.

49. The plant host system of claim 48, wherein the N-terminus of said expressed G-CSF is met-G-CSF.

50. The plant host system of claim 41, wherein said expressed cytokine is free from novel glycosylation.

51. A chimeric nucleic acid sequence capable of being expressed in a plant host system comprising:

a first nucleic acid sequence capable of regulating the transcription in said plant host system of a second nucleic acid sequence wherein said second nucleic acid sequence encodes a signal sequence is linked in reading frame to a third nucleic acid sequence encoding a cytokine.

52. The method of claim 51, wherein said nucleic acid sequence further comprises a fourth nucleic acid sequence linked in reading frame to the 3' end of said third nucleic acid sequence.

53. The method of claim 52, wherein said fourth nucleic acid sequence encodes a "KDEL" amino acid sequence.

54. The chimeric nucleic acid sequence of claim 51, wherein said first nucleic acid sequence comprises a plant active promoter.

55. The chimeric nucleic acid sequence of claim 51, wherein said signal sequence capable of targeting said cytokine to a sub-cellular location within said plant host system.

56. The chimeric nucleic acid sequence of claim 51, wherein said signal sequence is capable of targeting said cytokine to the cytosol of said plant host system.

57. The chimeric nucleic acid sequence of claim 51, wherein signal sequence is capable of targeting said cytokine to a plastid within said plant host system.

58. The chimeric nucleic acid sequence of claim 51, wherein said signal sequence is capable of targeting said cytokine to the endoplasmic reticulum located within said plant host system.

59. The chimeric nucleic acid sequence of claim 51, wherein said signal sequence comprises ubiquitin.

60. The method of claim 59, wherein said ubiquitin comprises an ubiquitin monomer derived from yeast.

61. The method of claim 59, wherein said ubiquitin comprises an ubiquitin monomer of potato ubiquitin gene 3.

62. The chimeric nucleic acid sequence of claim 51, wherein said signal sequence comprises a sufficient portion of oleosin to target said cytokine within said plant host system.

63. The chimeric nucleic acid sequence of claim 62, wherein a nucleic acid sequence encoding said oleosin is derived from soy.

64. The chimeric nucleic acid sequence of claim 51, wherein a nucleic acid sequence encoding an amino acid sequence that is specifically cleavable by enzymatic or chemical means is included between said signal sequence and said third nucleic acid sequence encoding a cytokine.

65. The chimeric nucleic acid sequence of claim 64, wherein said cleavable amino acid sequence comprises enterokinase.

66. The chimeric nucleic acid sequence of claim 64, wherein said signal sequence comprises a sufficient portion of oleosin protein to target said cytokine within said plant host system.

67. The chimeric nucleic acid sequence of claim 66, wherein a nucleic acid encoding said oleosin protein is derived from soy.

68. The chimeric nucleic acid sequence of claim 51, wherein cultivating said plant host system under the appropriate conditions results in the expression of said cytokine.

69. The chimeric nucleic acid sequence of claim 68, wherein said expressed cytokine is purified from said plant host system.

70. The chimeric nucleic acid sequence of claim 68, wherein said expressed cytokine is free from amino acid modifications.

71. The chimeric nucleic acid sequence of claim 70, wherein said amino acid modification comprises the addition of hydroxyproline to said cytokine.

72. The chimeric nucleic acid sequence of claim 68, wherein said expressed cytokine is a member of the cytokine superfamily selected from the group consisting of TGF-beta, PDGF, EGF, VEGF; chemokines; and FGFs.

73. The chimeric nucleic acid sequence of claim 72, wherein said expressed cytokine is hGH.

74. The chimeric nucleic acid sequence of claim 73, wherein the N-terminus of said expressed hGH is identical to the authentic N-terminus of hGH.

75. The chimeric nucleic acid sequence of claim 72, wherein said expressed cytokine comprises G-CSF.

76. The chimeric nucleic acid sequence of claim 75, wherein the N-terminus of said expressed G-CSF is met-G-CSF.

77. The chimeric nucleic acid sequence of claim 68, wherein said expressed cytokine is free from novel glycosylation.

78. An expression cassette comprising a chimeric nucleic acid sequence according to claim 51.

79. A plant transformed with a chimeric nucleic acid sequence according to claim 51.
80. A plant cell culture transformed with a chimeric nucleic acid sequence according to claim 51.
81. A plant seed containing a chimeric nucleic acid sequence according to claim 51.
82. A method of preparing a bioactive, authentic mammalian growth hormone in corn plants comprising the steps of
- (a) inserting a gene for said growth hormone into a corn plant expression vector;
 - (b) transforming corn plant cells with said expression vector;
 - (c) generating whole corn plants from said transformed corn cells;
 - (d) harvesting corn seed from whole corn plants; and
 - (e) purifying said growth hormone from corn seed.
83. The method of claim 82, wherein said mammalian growth hormone is human growth hormone.
84. The method of claim 82, wherein said growth hormone accumulates to a level greater than 1% of the total soluble protein in a plant sample.
85. The method of claim 84, wherein said growth hormone accumulates to level greater than 5% of the total soluble protein in a plant sample.
86. The method of claim 82, wherein said growth hormone is not glycosylated.
87. The method of claim 82, wherein said corn plant expression vector is pwrg4825.
88. Transformed corn plants and corn seed prepared by the method of claim 82.
89. A method of preparing bioactive, authentic human growth hormone from corn seed of claim 82, further comprising the steps of

- (a) extracting powdered corn seed with buffered saline, wherein said extraction is carried out at a pH ranging from about pH 8 to about pH 10;
- (b) adding urea to a concentration of about 2M to 3.5 M urea;
- (c) adjusting the pH of the extract to about pH 5;
- (d) clarifying the solution;
- (e) purifying by cation exchange chromatography, wherein said cation exchange chromatography is carried out in the presence of urea at a pH from about 4.5 to about 5.5; and
- (f) purifying by anion exchange chromatography, wherein said anion exchange chromatography is carried out in the absence of urea at a pH from about 7.0 to about 8.0.

90. A cytokine that is produced from a plant host system expressing a nucleic acid sequence wherein said nucleic acid sequence comprises

a first nucleic acid sequence capable of regulating the transcription in said plant host system of a second nucleic acid sequence wherein said nucleic acid sequence encodes a 5' regulatory region is linked in reading frame to a third nucleic acid sequence encoding a cytokine.

91. A method for producing a cytokine in a plant host system wherein said plant host system has been transformed with a chimeric nucleic acid sequence encoding a cytokine, comprising the step of:

cultivating said transformed plant host system under the appropriate conditions to result in expression of said cytokine, wherein said expressed cytokine is free from amino acid modifications in said plant host system.

92. A method for producing a cytokine in a plant host system wherein said plant host system has been transformed with a chimeric nucleic acid sequence encoding a cytokine, comprising the step of:

cultivating said transformed plant host system under the appropriate conditions to result in expression of said cytokine, wherein said expressed cytokine is free novel glycosylation in said plant host system.

ADD
A4